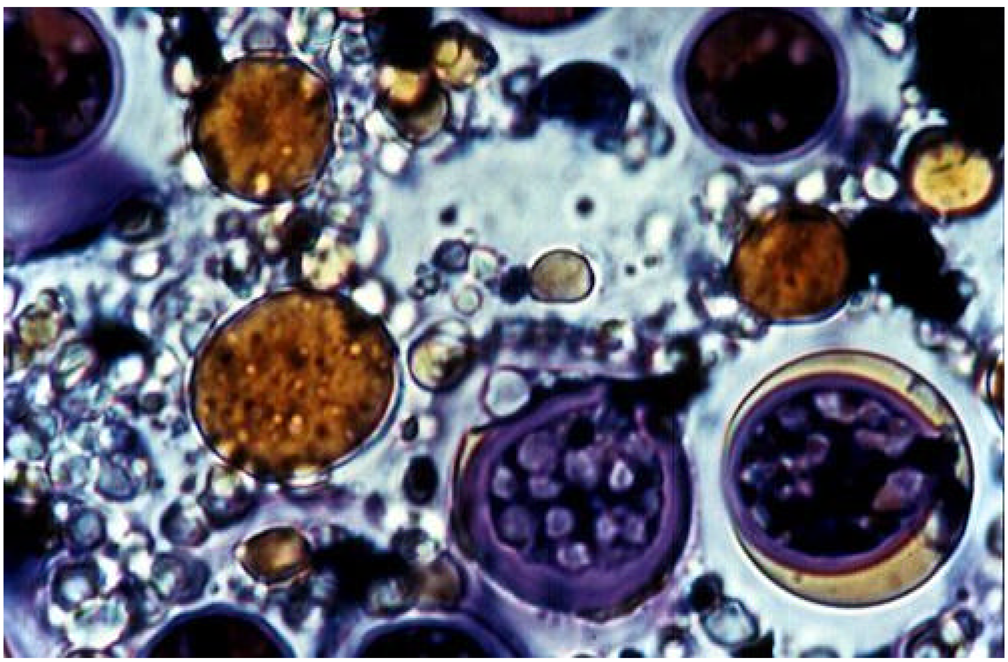
# A Look Back at the U.S. Department of Energy's Aquatic Species Program:



# Biodiesel from Algae

*Part I:*

*Program Summary*

# Background

## Origins of the Program

This year marks the 20th anniversary of the National Renewable Energy Laboratory (NREL). In 1978, the Carter Administration established what was then called the Solar Energy Research Institute (SERI) in Golden, CO. This was a first-of-its kind federal laboratory dedicated to the development of solar energy. The formation of this lab came in response to the energy crises of the early and mid 1970s. At the same time, the Carter Administration consolidated all federal energy activities under the auspices of the newly established U.S. Department of Energy (DOE).

Among its various programs established to develop all forms of solar energy, DOE initiated research on the use of plant life as a source of transportation fuels. Today, this program—known as the Biofuels Program—is funded and managed by the Office of Fuels Development (OFD) within the Office of Transportation Technologies under the Assistant Secretary for Energy Efficiency and Renewable Energy at DOE. The program has, over the years, focused on a broad range of alternative fuels, including ethanol and methanol (alcohol fuel substitutes for gasoline), biogas (methane derived from plant materials) and biodiesel (a natural oil-derived diesel fuel substitute). The Aquatic Species Program (ASP) was just one component of research within the Biofuels Program aimed at developing alternative sources of natural oil for biodiesel production.

## Close-out of the Program

The Aquatic Species Program (ASP) was a relatively small research effort intended to look at the use of aquatic plants as sources of energy. While its history dates back to 1978, much of the research from 1978 to 1982 was focused on using algae to produce hydrogen. The program switched emphasis to other transportation fuels, in particular biodiesel, beginning in the early 1980s. This report provides a summary of the research activities carried out from 1980 to 1996, with an emphasis on algae for biodiesel production.

In 1995, DOE made the difficult decision to eliminate funding for algae research within the Biofuels Program. Under pressure to reduce budgets, the Department chose a strategy of more narrowly focusing its limited resources in one or two key areas, the largest of these being the development of bioethanol. The purpose of this report is to bring closure to the Biofuels Program's algae research. This report is a summary and compilation of all the work done over the last 16 years of the program. It includes work carried out by NREL researchers at our labs in Golden, as well as subcontracted research and development activities conducted by private companies and universities around the country. More importantly, this report should be seen not as an ending, but as a beginning. When the time is right, we fully expect to see renewed interest in algae as a source of fuels and other chemicals. The highlights presented here should serve as a foundation for these future efforts.

## What is the technology?

### Biological Concepts

Photosynthetic organisms include plants, algae and some photosynthetic bacteria. Photosynthesis is the key to making solar energy available in useable forms for all organic life in our environment. These organisms use energy from the sun to combine water with carbon dioxide ($CO_2$) to create biomass. While other elements of the Biofuels Program have focused on terrestrial plants as sources of fuels, ASP was concerned with photosynthetic organisms that grew in aquatic environments. These include macroalgae, microalgae and emergents. Macroalgae, more commonly known as "seaweed," are fast growing marine and freshwater plants that can grow to considerable size (up to 60m in length). Emergents are plants that grow partially submerged in bogs and marshes. Microalgae are, as the name suggests, microscopic photosynthetic organisms. Like macroalgae, these organisms are found in both marine and freshwater environments. In the early days of the program, research was done on all three types of aquatic species. As emphasis switched to production of natural oils for biodiesel, microalgae became the exclusive focus of the research. This is because microalgae generally produce more of the right kinds of natural oils needed for biodiesel (see the discussion of fuel concepts presented later in this overview).

In many ways, the study of microalgae is a relatively limited field of study. Algae are not nearly as well understood as other organisms that have found a role in today's biotechnology industry. This is part of what makes our program so valuable. Much of the work done over the past two decades represents genuine additions to the scientific literature. The limited size of the scientific community involved in this work also makes it more difficult, and sometimes slower, compared to the progress seen with more conventional organisms. The study of microalgae represents an area of high risk and high gains.

These photosynthetic organisms are far from monolithic. Biologists have categorized microalgae in a variety of classes, mainly distinguished by their pigmentation, life cycle and basic cellular structure. The four most important (at least in terms of abundance) are:

- The diatoms (Bacillariophyceae). These algae dominate the phytoplankton of the oceans, but are also found in fresh and brackish water. Approximately 100,000 species are known to exist. Diatoms contain polymerized silica (Si) in their cell walls. All cells store carbon in a variety of forms. Diatoms store carbon in the form of natural oils or as a polymer of carbohydrates known as chyrsolaminarin.

- The green algae (Chlorophyceae). These are also quite abundant, especially in freshwater. (Anyone who owns a swimming pool is more than familiar with this class of algae). They can occur as single cells or as colonies. Green algae are the evolutionary progenitors of modern plants. The main storage compound for green algae is starch, though oils can be produced under certain conditions.

- The blue-green algae (Cyanophyceae). Much closer to bacteria in structure and organization, these algae play an important role in fixing nitrogen from the atmosphere. There are approximately 2,000 known species found in a variety of habitats.

- The golden algae (Chrysophyceae). This group of algae is similar to the diatoms. They have more complex pigment systems, and can appear yellow, brown or orange in color. Approximately 1,000 species are known to exist, primarily in freshwater systems. They are similar to diatoms in pigmentation and biochemical composition. The golden algae produce natural oils and carbohydrates as storage compounds.

The bulk of the organisms collected and studied in this program fall in the first two classes—the diatoms and the green algae.

Microalgae are the most primitive form of plants. While the mechanism of photosynthesis in microalgae is similar to that of higher plants, they are generally more efficient converters of solar energy because of their simple cellular structure. In addition, because the cells grow in aqueous suspension, they have more efficient access to water, $CO_2$, and other nutrients. For these reasons, microalgae are capable of producing 30 times the amount oil per unit area of land, compared to terrestrial oilseed crops.

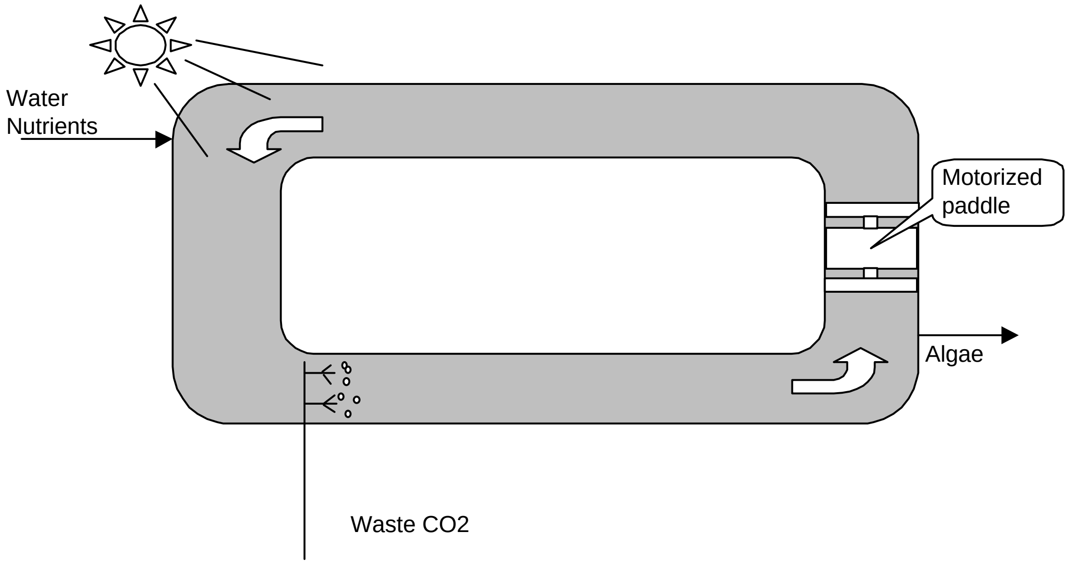
*Put quite simply, microalgae are remarkable and efficient biological factories capable of taking a waste (zero-energy) form of carbon ($CO_2$) and converting it into a high density liquid form of energy (natural oil). This ability has been the foundation of the research program funded by the Office Fuels Development.*

### Algae Production Concepts

Like many good ideas (and certainly many of the concepts that are now the basis for renewable energy technology), the concept of using microalgae as a source of fuel is older than most people realize. The idea of producing methane gas from algae was proposed in the early 1950s[1]. These early researchers visualized a process in which wastewater could be used as a medium and source of nutrients for algae production. The concept found a new life with the energy crisis of the 1970s. DOE and its predecessors funded work on this combined process for wastewater treatment and energy production during the 1970s. This approach had the benefit of serving multiple needs—both environmental and energy-related. It was seen as a way of introducing this alternative energy source in a near-term timeframe.

In the 1980s, DOE's program gradually shifted its focus to technologies that could have large-scale impacts on national consumption of fossil energy. Much of DOE's publications from this period reflect a philosophy of energy research that might, somewhat pejoratively, be called "the quads mentality." A quad is a short-hand name for the unit of energy often used by DOE to describe the amounts of energy that a given technology might be able to displace. Quad is short for "quadrillion Btus"—a unit of energy representing $10^{15}$ (1,000,000,000,000,000) Btus of energy. This perspective led DOE to focus on the concept of immense algae farms.

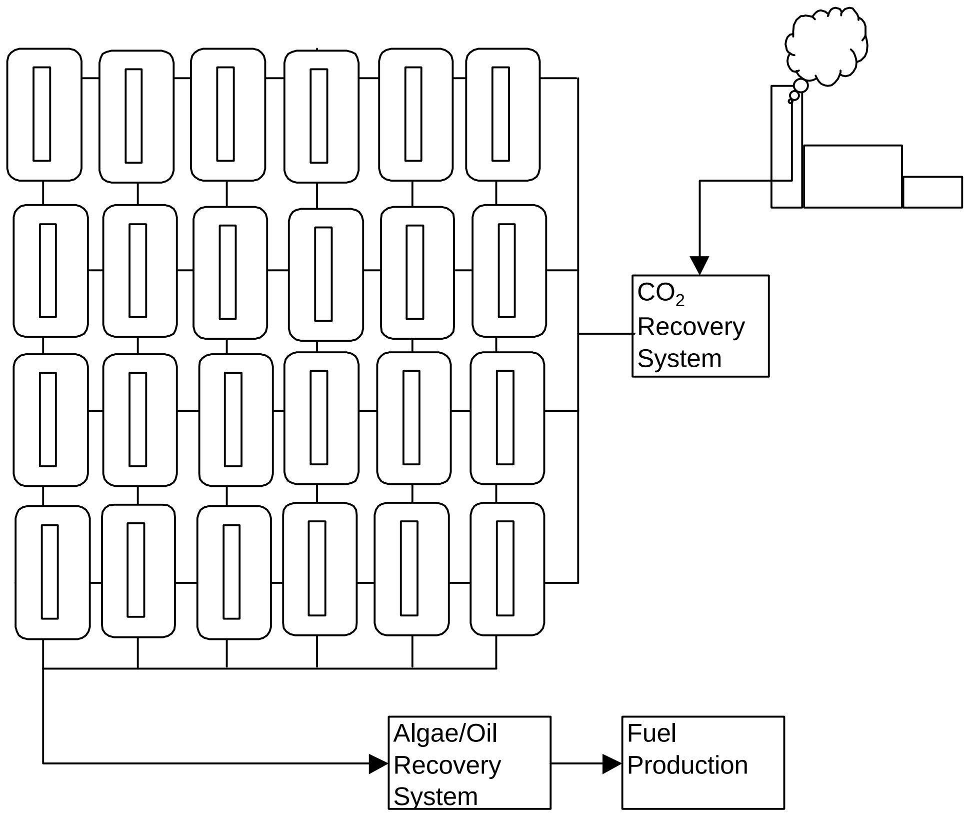
Such algae farms would be based on the use of open, shallow ponds in which some source of waste $CO_2$ could be efficiently bubbled into the ponds and captured by the algae (see the figure below).




The ponds are "raceway" designs, in which the algae, water and nutrients circulate around a racetrack. Paddlewheels provide the flow. The algae are thus kept suspended in water. Algae are circulated back up to the surface on a regular frequency. The ponds are kept shallow because of the need to keep the algae exposed to sunlight and the limited depth to which sunlight can penetrate the pond water. The ponds are operated continuously; that is, water and nutrients are constantly fed to the pond, while algae-containing water is removed at the other end. Some kind of harvesting system is required to recover the algae, which contains substantial amounts of natural oil.

The concept of an "algae farm" is illustrated on the next page. The size of these ponds is measured in terms of surface area (as opposed to volume), since surface area is so critical to capturing sunlight. Their productivity is measured in terms of biomass produced per day per unit of available surface area. Even at levels of productivity that would stretch the limits of an aggressive research and development program, such systems will require acres of land. At such large sizes, it is more appropriate to think of these operations on the scale of a farm.

There are quite a number of sources of waste $CO_2$. Every operation that involves combustion of fuel for energy is a potential source. The program targeted coal and other fossil fuel-fired power plants as the main sources of $CO_2$. Typical coal-fired power plants emit flue gas from their stacks containing up to 13% $CO_2$. This high concentration of $CO_2$ enhances transfer and uptake of $CO_2$ in the ponds. The concept of coupling a coal-fired power plant with an algae farm provides an elegant approach to recycle of the $CO_2$ from coal combustion into a useable liquid fuel.




Other system designs are possible. The Japanese, French and German governments have invested significant R&D dollars on novel closed bioreactor designs for algae production. The main advantage of such closed systems is that they are not as subject to contamination with whatever organism happens to be carried in the wind. The Japanese have, for example, developed optical fiber-based reactor systems that could dramatically reduce the amount of surface area required for algae production. While breakthroughs in these types of systems may well occur, their costs are, for now, prohibitive—especially for production of fuels. DOE's program focused primarily on open pond raceway systems because of their relative low cost.

*The Aquatic Species Program envisioned vast arrays of algae ponds covering acres of land analogous to traditional farming. Such large farms would be located adjacent to power plants. The bubbling of flue gas from a power plant into these ponds provides a system for recycling of waste $CO_2$ from the burning of fossil fuels.*

### Fuel Production Concepts

The previous sections have alluded to a number of potential fuel products from algae. The ASP considered three main options for fuel production:

- Production of methane gas via biological or thermal gasification.
- Production of ethanol via fermentation

- Production of biodiesel

A fourth option is the direct combustion of the algal biomass for production of steam or electricity. Because the Office of Fuels Development has a mandate to work on transportation fuels, the ASP did not focus much attention on direct combustion. The concept of algal biomass as a fuel extender in coal-fired power plants was evaluated under a separate program funded by DOE's Office of Fossil Fuels. The Japanese have been the most aggressive in pursuing this application. They have sponsored demonstrations of algae production and use at a Japanese power plant.

Algal biomass contains three main components:

- Carbohydrates
- Protein
- Natural Oils

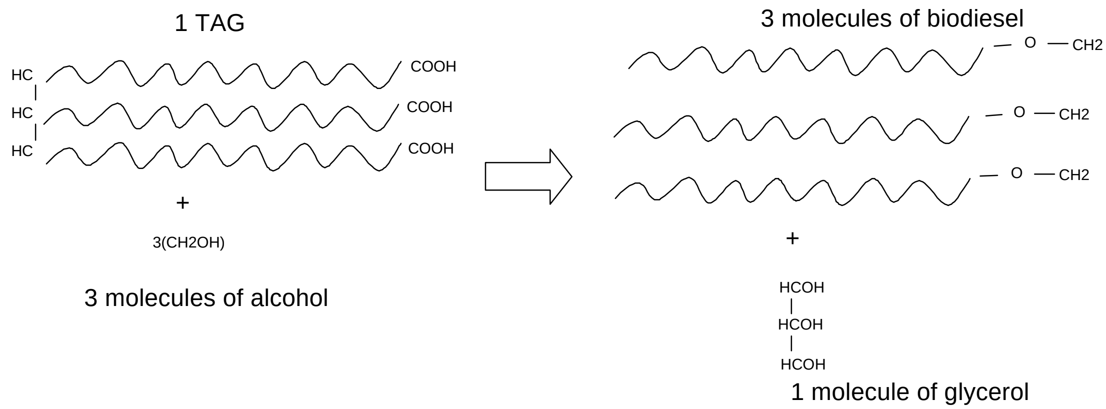
The economics of fuel production from algae (or from any biomass, for that matter) demands that we utilize all the biomass as efficiently as possible. To achieve this, the three fuel production options listed previously can be used in a number of combinations. The most simplistic approach is to produce methane gas, since the both the biological and thermal processes involved are not very sensitive to what form the biomass is in. Gasification is a somewhat brute force technology in the sense that it involves the breakdown of any form of organic carbon into methane. Ethanol production, by contrast, is most effective for conversion of the carbohydrate fraction. Biodiesel production applies exclusively to the natural oil fraction. Some combination of all three components can also be utilized as an animal feed. Process design models developed under the program considered a combination of animal feed production, biological gasification and biodiesel production.

The main product of interest in the ASP was biodiesel. In its most general sense, biodiesel is any biomass-derived diesel fuel substitute. Today, biodiesel has come to mean a very specific chemical modification of natural oils. Oilseed crops such as rapeseed (in Europe) and soybean oil (in the U.S.) have been extensively evaluated as sources of biodiesel. Biodiesel made from rapeseed oil is now a substantial commercial enterprise in Europe. Commercialization of biodiesel in the U.S. is still in its nascent stage.

The bulk of the natural oil made by oilseed crops is in the form of triacylglycerols (TAGs). TAGs consist of three long chains of fatty acids attached to a glycerol backbone. The algae species studied in this program can produce up to 60% of their body weight in the form of TAGs. Thus, algae represent an alternative source of biodiesel, one that does not compete with the existing oilseed market.

As a matter of historical interest, Rudolph Diesel first used peanut oil (which is mostly in the form of TAGs) at the turn of the century to demonstrate his patented diesel engine[2]. The rapid introduction of cheap petroleum quickly made petroleum the preferred source of diesel fuel, so much so that today's diesel engines do not operate well when operated on unmodified TAGs. Natural oils, it turns out, are too viscous to be used in modern diesel engines.

In the 1980s, a chemical modification of natural oils was introduced that helped to bring the viscosity of the oils within the range of current petroleum diesel[3]. By reacting these TAGs with simple alcohols (a chemical reaction known as "transesterification" already commonplace in the oleochemicals industry), we can create a chemical compound known as an alkyl ester[4], but which is known more generically as biodiesel (see the figure below). Its properties are very close to those of petroleum diesel fuel.




Commercial experience with biodiesel has been very promising[5]. Biodiesel performs as well as petroleum diesel, while reducing emissions of particulate matter, CO, hydrocarbons and $SO_x$. Emissions of $NO_x$ are, however, higher for biodiesel in many engines. Biodiesel virtually eliminates the notorious black soot emissions associated with diesel engines. Total particulate matter emissions are also much lower[6,7,8]. Other environmental benefits of biodiesel include the fact that it is highly biodegradable[9] and that it appears to reduce emissions of air toxics and carcinogens (relative to petroleum diesel)[10]. A proper discussion of biodiesel would require much more space than can be accommodated here. Suffice it to say that, given many of its environmental benefits and the emerging success of the fuel in Europe, biodiesel is a very promising fuel product.

*High oil-producing algae can be used to produce biodiesel, a chemically modified natural oil that is emerging as an exciting new option for diesel engines. At the same time, algae technology provides a means for recycling waste carbon from fossil fuel combustion. Algal biodiesel is one of the only avenues available for high-volume re-use of $CO_2$ generated in power plants. It is a technology that marries the potential need for carbon disposal in the electric utility industry with the need for clean-burning alternatives to petroleum in the transportation sector.*

## Why microalgae technology?

There are a number of benefits that serve as driving forces for developing and deploying algae technology. Some of these benefits have already been mentioned. Four key areas are outlined here. The first two address national and international issues that continue to grow in importance—energy security and climate change. The remaining areas address aspects of algae technology that differentiate it from other technology options being pursued by DOE.

### Energy Security

Energy security is the number one driving force behind DOE's Biofuels Program. The U.S. transportation sector is at the heart of this security issue. Cheap oil prices during the 1980s and 1990s have driven foreign oil imports to all time highs. In 1996, imports reached an important milestone—imported oil consumption exceeded domestic oil consumption. DOE's Energy Information Administration paints a dismal picture of our growing dependence on foreign oil. Consider these basic points[11]:

- Petroleum demand is increasing, especially due to new demand from Asian markets.
- New demand for oil will come primarily from the Persian Gulf.
- As long as prices for petroleum remain low, we can expect our imports to exceed 60% of our total consumption ten years from now.
- U.S. domestic supplies will likewise remain low as long as prices for petroleum remain low.

Not everyone shares this view of the future, or sees it as a reason for concern. The American Petroleum Institute[12] does not see foreign imports as a matter of national security. Others have argued that the prediction of increasing Mideast oil dependence worldwide is wrong. But the concern about our foreign oil addiction is widely held by a broad range of political and commercial perspectives[13].

While there may be uncertainty and even contention over when and if there is a national security issue, there is one more piece to the puzzle that influences our perspective on this issue. This is the fact that, quite simply, 98% of the transportation sector in the U.S. relies on petroleum (mostly in the form of gasoline and diesel fuel). The implication of this indisputable observation is that even minor hiccups in the supply of oil could have crippling effects on our nation. This lends special significance to the Biofuels Program as a means of diversifying the fuel base in our transportation sector.

> *Our almost complete reliance on petroleum in transportation comes from the demand for gasoline in passenger vehicles and the demand for diesel fuel in commerce. Bioethanol made from terrestrial energy crops offers a future alternative to gasoline, biodiesel made from algal oils could do the same for diesel fuel.*

### Climate Change

$CO_2$ is recognized as the most important (at least in quantity) of the atmospheric pollutants that contribute to the "greenhouse effect," a term coined by the French mathematician Fourier in the mid-1800s to describe the trapping of heat in the Earth's atmosphere by gases capable of absorbing radiation. By the end of the last century, scientists were already speculating on the potential impacts of anthropogenic

$CO_2$. The watershed event that brought the question of global warming to the forefront in the scientific community was the publication of Revelle's data in 1957, which quantified the geologically unprecedented build-up of atmospheric $CO_2$ that began with the advent of the industrial revolution. Revelle[14] characterized the potential risk of global climate change this way:

> *"Human beings are carrying out a large scale geophysical experiment of a kind that could not have happened in the past nor be produced in the future. Within a few centuries, we are returning to the atmosphere and the oceans the concentrated organic carbon stored in sedimentary rocks over hundreds of millions of years."*

Despite 40 years of research since Revelle first identified the potential risk of global warming, the debate over the real impacts of the increased $CO_2$ levels still rages. We may never be able to scientifically predict the climatic effects of increasing carbon dioxide levels due to the complexity of atmospheric and meteorological modeling. Indeed, Revelle's concise statement of the risks at play in global climate change remains the best framing of the issue available for policy makers today. The question we face as a nation is how much risk we are willing to take on an issue like this. That debate has never properly taken place with the American public.

> *As Revelle's statement implies, the burning of fossil fuels is the major source of the current build up of atmospheric $CO_2$. Thus, identifying alternatives to fossil fuels must be a key strategy in reducing greenhouse gas emissions. While no one single fuel can substitute for fossil fuels in an all of the energy sectors, we believe that biodiesel made from algal oils is a fuel which can make a major contribution to the reduction of $CO_2$ generated by power plants and commercial diesel engines.*

### The Synergy of Coal and Microalgae

Many of our fossil fuel reserves, but especially coal, are going to play significant roles for years to come. On a worldwide basis, coal is, by far, the largest fossil energy resource available. About one-fourth of the world's coal reserves reside in the United States. To put this in perspective, consider the fact that, at current rates of consumption, coal reserves could last for over 200 years.

Regardless of how much faith you put in future fossil energy projections, it is clear that coal will continue to play an important role in our energy future—especially given the relatively large amounts of coal that we control within our own borders. DOE's Energy Information Administration estimates that electricity will become an increasingly large contributor to future U.S. energy demand. How will this new demand be met? Initially, low cost natural gas will grow in use. Inevitably, the demand for electricity will have to be met by coal. Coal will remain the mainstay of U.S. baseline electricity generation, accounting for half of electricity generation by the year 2010.

The long term demand for coal brings with it a demand for technologies that can mitigate the environmental problems associated with coal. While control technologies will be used to reduce air pollutants associated with acid rain, no technologies exist today which address the problem of greenhouse gas emissions. Coal is the most carbon-intensive of the fossil fuels. In other words, for every Btu of energy liberated by combustion, coal emits more $CO_2$ than either petroleum or natural gas. As pressure to reduce carbon emissions grows, this will become an increasingly acute problem for the U.S.

One measure of how serious this problem could be is the absurdity of some of the proposals being developed for handling carbon emissions from power plants. The preferred option offered by researchers at MIT is ocean disposal, despite the expense and uncertainty of piping $CO_2$ from power plants and injecting the $CO_2$ in the ocean[15].

Commonsense suggests that recycling of carbon would be more efficacious than deep ocean disposal. No one clearly understands the long-term effects of injecting large amounts of $CO_2$ into our oceans. Beyond these environmental concerns, such large-scale disposal schemes represent an economic sinkhole. Huge amounts of capital and operating dollars would be spent simply to dispose of carbon. While such Draconian measures may ultimately be needed, it makes more sense to first re-use stationary sources of carbon as much as possible. Algae technology is unique in its ability to produce a useful, high-volume product from waste $CO_2$.

*Consumption of coal, an abundant domestic fuel source for electricity generation, will continue to grow over the coming decades, both in the U.S. and abroad. Algae technology can extend the useful energy we get from coal combustion and reduce carbon emissions by recycling waste $CO_2$ from power plants into clean-burning biodiesel. When compared to the extreme measures proposed for disposing of power plant carbon emissions, algal recycling of carbon simply makes sense.*

### Terrestrial versus Aquatic Biomass

Algae grow in aquatic environments. In that sense, algae technology will not compete for the land already being eyed by proponents of other biomass-based fuel technologies. Biomass power and bioethanol both compete for the same land and for similar feedstocks—trees and grasses specifically grown for energy production. More importantly, many of the algal species studied in this program can grow in brackish water—that is, water that contains high levels of salt. This means that algae technology will not put additional demand on freshwater supplies needed for domestic, industrial and agricultural use.

The unique ability of algae to grow in saline water means that we can target areas of the country in which saline groundwater supplies prevent any other useful application of water or land resources. If we were to draw a map showing areas best suited for energy crop production (based on climate and resource needs), we would see that algae technology needs *complement* the needs of both agriculture and other biomass-based energy technologies.

*In a world of ever more limited natural resources, algae technology offers the opportunity to utilize land and water resources that are, today, unsuited for any other use. Land use needs for microalgae complement, rather than compete, with other biomass-based fuel technologies.*

## Technical Highlights of the Program

### Applied Biology

*A unique collection of oil-producing microalgae.*

The ASP studied a fairly specific aspect of algae—their ability to produce natural oils. Researchers not only concerned themselves with finding algae that produced a lot of oil, but also with algae that grow under severe conditions—extremes of temperature, pH and salinity. At the outset of the program, no collections existed that either emphasized or characterized algae in terms of these constraints. Early on, researchers set out to build such a collection. Algae were collected from sites in the west, the northwest and the southeastern regions of the continental U.S., as well as Hawaii. At its peak, the collection contained over 3,000 strains of organisms. After screening, isolation and characterization efforts, the collection was eventually winnowed down to around 300 species, mostly green algae and diatoms. The collection, now housed at the University of Hawaii, is still available to researchers. This collection is an untapped resource, both in terms of the unique organisms available and the mostly untapped genetic resource they represent. It is our sincere hope that future researchers will make use of the collection not only as a source of new products for energy production, but for many as yet undiscovered new products and genes for industry and medicine.

*Shedding light on the physiology and biochemistry of algae.*

Prior to this program, little work had been done to improve oil production in algal organisms. Much of the program's research focused attention on the elusive "lipid trigger." (Lipids are another generic name for TAGs, the primary storage form of natural oils.) This "trigger" refers to the observation that, under environmental stress, many microalgae appeared to flip a switch to turn on production of TAGs. Nutrient deficiency was the major factor studied. Our work with nitrogen-deficiency in algae and silicon deficiency in diatoms did not turn up any overwhelming evidence in support of this trigger theory. The common thread among the studies showing increased oil production under stress seems to be the observed cessation of cell division. While the rate of production of all cell components is lower under nutrient starvation, oil production seems to remain higher, leading to an accumulation of oil in the cells. The increased oil content of the algae does not to lead to increased overall productivity of oil. In fact, overall rates of oil production are lower during periods of nutrient deficiency. Higher levels of oil in the cells are more than offset by lower rates of cell growth.

*Breakthroughs in molecular biology and genetic engineering.*

Plant biotechnology is a field that is only now coming into its own. Within the field of plant biotechnology, algae research is one of the least trodden territories. The slower rate of advance in this field makes each step forward in our research all the more remarkable. Our work on the molecular biology and genetics of algae is thus marked with significant scientific discoveries. The program was the first to isolate the enzyme Acetyl CoA Carboxylase (ACCase) from a diatom. This enzyme was found to catalyze a key metabolic step in the synthesis of oils in algae. The gene that encodes for the production of ACCase was eventually isolated and cloned. This was the *first* report of the cloning of the full sequence of the ACCase gene in *any* photosynthetic organism. With this gene in hand, researchers went on to develop the first successful transformation system for diatoms—the tools and genetic components for expressing a foreign gene. The ACCase gene and the transformation system for diatoms have both been patented. In the closing days of the program, researchers initiated the first experiments in metabolic engineering as a means of increasing oil production. Researchers demonstrated an ability to make algae over-express the ACCase gene, a major milestone for the research, with the hope that increasing the level of ACCase activity in the cells would lead to higher oil production. These early experiments did not, however, demonstrate increased oil production in the cells.

### Algae Production Systems

*Demonstration of Open Pond Systems for Mass Production of Microalgae.*

Over the course of the program, efforts were made to establish the feasibility of large-scale algae production in open ponds. In studies conducted in California, Hawaii and New Mexico, the ASP proved the concept of long term, reliable production of algae. California and Hawaii served as early test bed sites. Based on results from six years of tests run in parallel in California and Hawaii, 1,000 $m^2$ pond systems were built and tested in Roswell, New Mexico. The Roswell, New Mexico tests proved that outdoor ponds could be run with extremely high efficiency of $CO_2$ utilization. Careful control of pH and other physical conditions for introducing $CO_2$ into the ponds allowed greater than 90% utilization of injected $CO_2$. The Roswell test site successfully completed a full year of operation with reasonable control of the algal species grown. Single day productivities reported over the course of one year were as high as 50 grams of algae per square meter per day, a long-term target for the program. Attempts to achieve consistently high productivities were hampered by low temperature conditions encountered at the site. The desert conditions of New Mexico provided ample sunlight, but temperatures regularly reached low levels (especially at night). If such locations are to be used in the future, some form of temperature control with enclosure of the ponds may well be required.

*A disconnect between the lab and the field.*

An important lesson from the outdoor testing of algae production systems is the inability to maintain laboratory organisms in the field. Algal species that looked very promising when tested in the laboratory were not robust under conditions encountered in the field. In fact, the best approach for successful cultivation of a consistent species of algae was to allow a contaminant native to the area to take over the ponds.

*The high cost of algae production remains an obstacle.*

The cost analyses for large-scale microalgae production evolved from rather superficial analyses in the 1970s to the much more detailed and sophisticated studies conducted during the 1980s. A major conclusion from these analyses is that there is little prospect for any alternatives to the open pond designs, given the low cost requirements associated with fuel production. The factors that most influence cost are biological, and not engineering-related. These analyses point to the need for highly productive organisms capable of near-theoretical levels of conversion of sunlight to biomass. Even with aggressive assumptions about biological productivity, we project costs for biodiesel which are two times higher than current petroleum diesel fuel costs.

### Resource Availability

*Land, water and $CO_2$ resources can support substantial biodiesel production and CO2 savings.*

The ASP regularly revisited the question of available resources for producing biodiesel from microalgae. This is not a trivial effort. Such resource assessments require a combined evaluation of appropriate climate, land and resource availability. These analyses indicate that significant potential land, water and $CO_2$ resources exist to support this technology. Algal biodiesel could easily supply several "quads" of biodiesel—substantially more than existing oilseed crops could provide. Microalgae systems use far less water than traditional oilseed crops. Land is hardly a limitation. Two hundred thousand hectares (less than 0.1% of climatically suitable land areas in the U.S.) could produce one quad of fuel. Thus, though the technology faces many R&D hurdles before it can be practicable, it is clear that resource limitations are not an argument against the technology.

## A Brief Chronology of the Research Activities

Part II of this report details the specific research accomplishments of the program on a year-to-year basis. In order to provide a consistent context and framework for understanding this detail, we have attempted to outline the major activities of the program as they unfolded over the course of the past two decades. The timeline on the following page shows the major activities broken down into two main categories—laboratory studies and outdoor testing/systems analysis. For the sake of clarity and brevity, many of the research projects and findings from the program are not presented here. Instead, only those findings that form a thread throughout the work are highlighted. There were many other studies and findings of value in the program. The reader is encouraged to review Part II of this report for a more comprehensive discussion of the research.

### Laboratory Studies

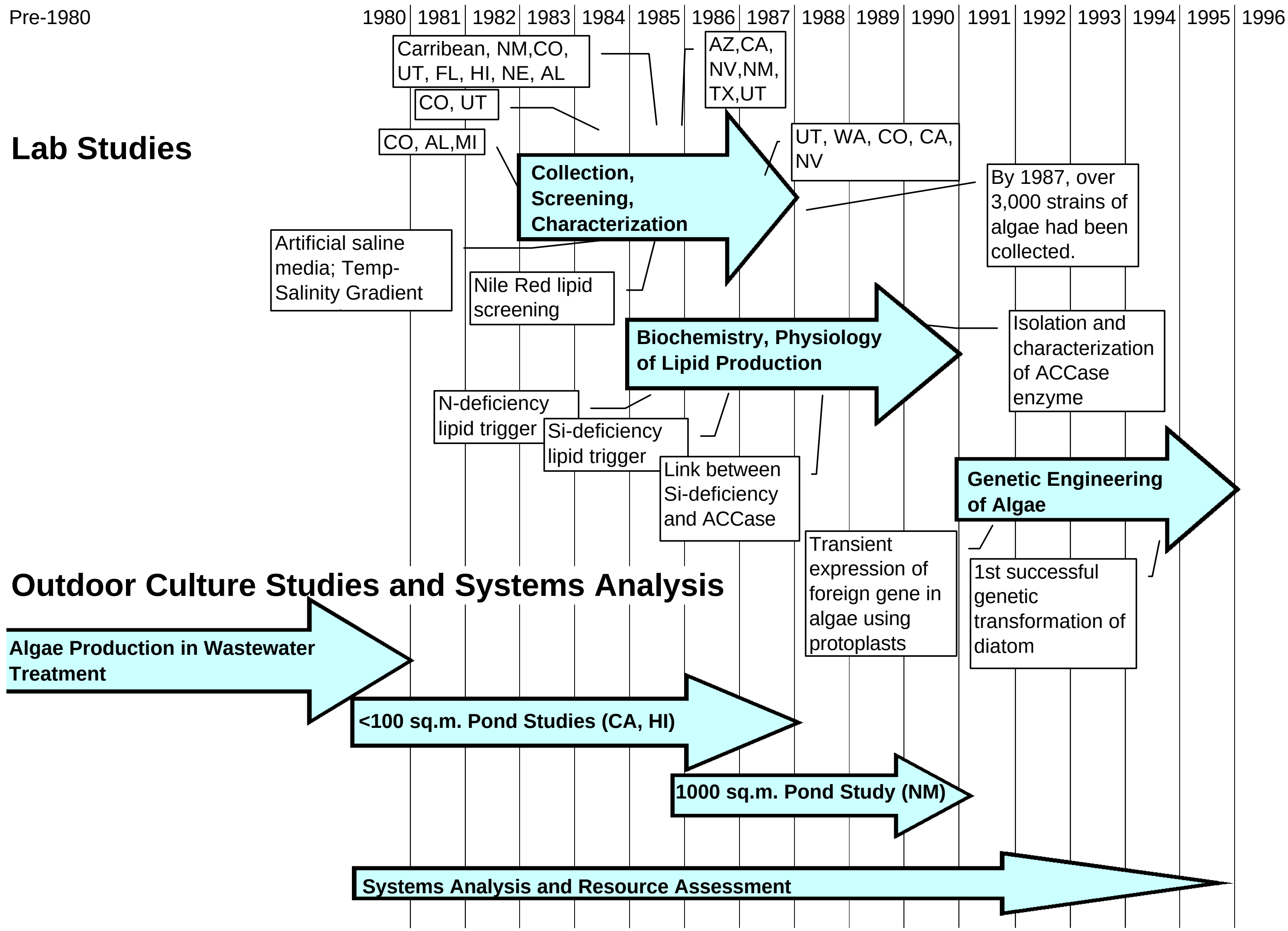
The research pathway in the lab can be broken down into three types of activities:

- Collection, screening and characterization of algae.
- Biochemical and physiological studies of lipid production
- Molecular biology and genetic engineering studies

There is a logic to the sequence of these activities. Researchers first identified a need to collect and identify algae that met minimal requirements for this technology. Collection and screening occurred over a seven-year period from 1980 to 1987. Once a substantial amount of information was available on the types of oil-producing algae and their capabilities, the program began to switch its emphasis to understanding the biochemistry and physiology of oil production in algae. A natural next step was to use this information to identify approaches to genetically manipulate the metabolism of algae to enhance oil production.

Algae collection efforts initially focused on shallow, inland saline habitats, particularly in western Colorado, New Mexico and Utah. The reasoning behind collecting strains from these habitats was that the strains would be adapted to at least some of the environmental conditions expected in mass culture facilities located in the southwestern U.S. (a region identified early on as a target for deployment of the technology). Organisms isolated from shallow habitats were also expected to be more tolerant to wide swings in temperature and salinity. In the meantime, subcontractors were collecting organisms from the southeastern region of the U.S. (Florida, Mississippi, and Alabama). By 1984, researchers in the program had developed improved tools and techniques for collecting and screening organisms. These included a modified rotary screening apparatus and statistically designed saline media formulations that mimicked typical brackish water conditions in the southwest. In 1985, a rapid screening test was in place for identifying high oil-producing algae. In the last years of the collection effort, the focus switched to finding algae that were tolerant to low temperature. This expanded the reach of the collection activities into the northwest. By 1987, the algae collection contained over 3,000 species.

As the collection efforts began to wind down, it became apparent that no one single species was going to be found that met all of the needs of the technology. As a result, about midway through the collection efforts, the program began studies on the biochemistry and physiology of oil production in algae in hopes of learning how to improve the performance of existing organisms. A number of ASP subcontractors struggled to identify the so-called "lipid trigger." These studies confirmed observations that deficiencies in nitrogen could lead to an increase in the level of oil present in many species of algae. Observations of cellular structure also supported the notion of a trigger that caused rapid build up of oil droplets in the cells during periods of nitrogen depletion.



Pre-1980
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
Lab Studies
Carribean, NM,CO, UT, FL, HI, NE, AL
CO, UT
CO, AL,MI
AZ,CA, NV,NM, TX,UT
UT, WA, CO, CA, NV
Collection, Screening, Characterization
By 1987, over 3,000 strains of algae had been collected.
Artificial saline media; Temp-Salinity Gradient
Nile Red lipid screening
Biochemistry, Physiology of Lipid Production
Isolation and characterization of ACCase enzyme
N-deficiency lipid trigger
Si-deficiency lipid trigger
Link between Si-deficiency and ACCase
Genetic Engineering of Algae
Transient expression of foreign gene in algae using protoplasts
1st successful genetic transformation of diatom
Outdoor Culture Studies and Systems Analysis
Algae Production in Wastewater Treatment
<100 sq.m. Pond Studies (CA, HI)
1000 sq.m. Pond Study (NM)
Systems Analysis and Resource Assessment

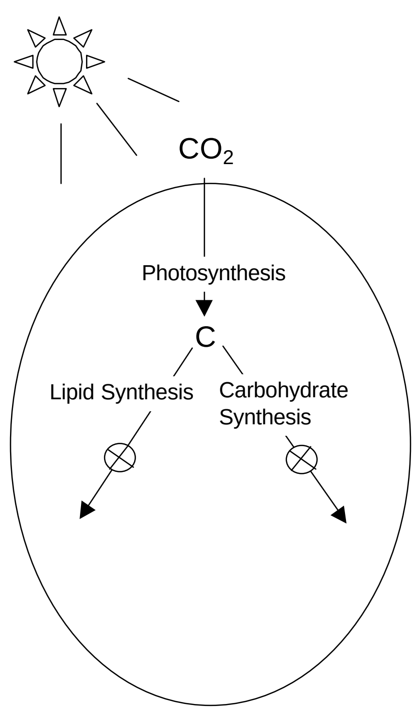
In the end, however, the studies conducted both by NREL researchers and program subcontractors concluded that no simple trigger for lipid production exists. Instead, we found that environmental stresses like nitrogen depletion lead to inhibition of cell division, without immediately slowing down oil production. It appeared that no simple means existed for increasing oil production, without a penalty in overall productivity due to a slowing down of cell growth. The use of nutrient depletion as a means of inducing oil production may still have merit. Some experiments conducted at NREL suggested that the kinetics of cell growth and lipid accumulation are very subtle. With a better understanding of these kinetics, it may be possible to provide a net increase in total oil productivity by carefully controlling the timing of nutrient depletion and cell harvesting.

In 1986, researchers at NREL reported on the use of Si depletion as a way to increase oil levels in diatoms. They found that when Si was used up, cell division slowed down since Si is a component of the diatoms' cell walls. In the diatom *C. cryptica*, the rate of oil production remained constant once Si depletion occurred, while growth rate of the cells dropped. Further studies identified two factors that seemed to be at play in this species:

1. Si-depleted cells direct newly assimilated carbon more toward lipid production and less toward carbohydrate production.

2. Si-depleted cells slowly convert non-lipid cell components to lipids.

Diatoms store carbon in lipid form or in carbohydrate form. The results of these experiments suggested that it might be possible to alter which route the cells used for storage (see schematic below):



Through the process of photosynthesis, algae cells assimilate carbon. There are numerous metabolic pathways through which the carbon can go, resulting in synthesis of whatever compounds are needed by the cell. These pathways consist of sequences of enzymes, each of which catalyzes a specific reaction. Two possible pathways for carbon are shown on the previous page. They represent the two storage forms that carbon can take.

Researchers at NREL began to look for key enzymes in the lipid synthesis pathway. These would be enzymes whose level of activity in the cell influences the rate at which oils are formed. Think of these enzymes as valves or spigots controlling the flow of carbon down the pathway. Higher enzyme activity leads to higher rates of oil production. When algae cells increase the activity of active enzymes, they are opening up the spigot to allow greater flow of carbon to oil production. Finding such critical enzymes was key to understanding the mechanisms for controlling oil production.

By 1988, researchers had shown that increases in the levels of the enzyme Acetyl CoA Carboxylase (ACCase) correlated well with lipid accumulation during Si depletion. They also showed that the increased levels correlated with increased expression of the gene encoding for this enzyme. These findings led to a focus on isolating the enzyme and cloning the gene responsible for its expression. By the end of the program, not only had researchers successfully cloned the ACCase gene, but they had also succeeded in developing the tools for expressing foreign genes in diatoms.

In the 1990s, genetic engineering had become the main focus of the program. While we have highlighted the successes of over-expressing ACCase in diatoms, other approaches were also developed for foreign gene expression—in green algae as well as in diatoms. Another interesting sideline in the research involved studies aimed at identifying key enzymes involved in the synthesis of storage carbohydrates. Instead of over-expressing these enzymes, researchers hoped to inactivate them. Returning to our “spigot” analogy, this approach was like shutting off the flow of carbon to carbohydrates, in the hopes that it would force carbon to flow down the lipid synthesis pathway (again, see the schematic on the previous page). This work led to the discovery of a unique multifunctional enzyme in the carbohydrate synthesis pathway. This enzyme and its gene were both patented by NREL in 1996.

### Outdoor Testing and Systems Analysis

The first work done in earnest by DOE on demonstration of algae technology for energy production predates the Aquatic Species Program. In 1976, the Energy Research and Development Administration (before it was folded into DOE) funded a project at the University of California Berkeley’s Richmond Field Station to evaluate a combined wastewater treatment/fuel production system based on microalgae. Over the course of several years, the Richmond Field Station demonstrated techniques for algae harvesting and for control of species growing in open ponds.

By the time the Aquatic Species Program took on microalgae research, emphasis had already moved from wastewater treatment based systems to dedicated algae farm operations. From 1980 to 1987, the program funded two parallel efforts to develop large scale mass culture systems for microalgae. One effort was at the University of California, and it was based on a so-called “High Rate Pond” (HRP) design. The other effort was carried out at the University of Hawaii, where a patented “Algae

Raceway Production System" (ARPS). Both designs utilized open raceway designs. The HRP design was based on a shallow, mixed raceway concept developed at Berkeley in 1963 and successfully applied in wastewater treatment operations in California. The ARPS was really a variation on the same concept. Both efforts carried out their test work in ponds of 100 square meters or less. They studied a variety of fundamental operational issues, such as the effects of fluid flow patterns, light intensity, dissolved oxygen levels, pH and algae harvesting methods.

At the conclusion of the smaller scale tests conducted in California and Hawaii, the program engaged in a competitive bidding process to select a system design for scale up of algae mass culture. The HRP design evaluated at UC Berkeley was selected for scale-up. The "Outdoor Test Facility" (OTF) was designed and built at the site of an abandoned water treatment plant in Roswell, New Mexico. From 1988 to 1990, 1,000 square meter ponds were successfully operated at Roswell. This project demonstrated how to achieve very efficient (>90%) utilization of $CO_2$ in large ponds. The best results were obtained using native species of algae that naturally took over in the ponds (as opposed to using laboratory cultures). The OTF also demonstrated production of high levels of oil in algae using both nitrogen and silica depletion strategies. While daily productivities did reach program target levels of 50 grams per square per day, overall productivity was much lower (around 10 grams per square meter per day) due to the number of cold temperature days encountered at this site. Nevertheless, the project established the proof-of-concept for large scale open pond operations. The facility was shut down in 1990, and has not been operated since.

A variety of other outdoor projects were funded over the course of the program, including a three-year project on algal biodiesel production conducted in Israel. In addition, research at the Georgia Institute of Technology was carried out in the late 1980s. This work consisted of a combination of experimental and computer modeling work. This project resulted in the development of the APM (Algal Pond Model), a computer modeling tool for predicting performance of outdoor pond systems.

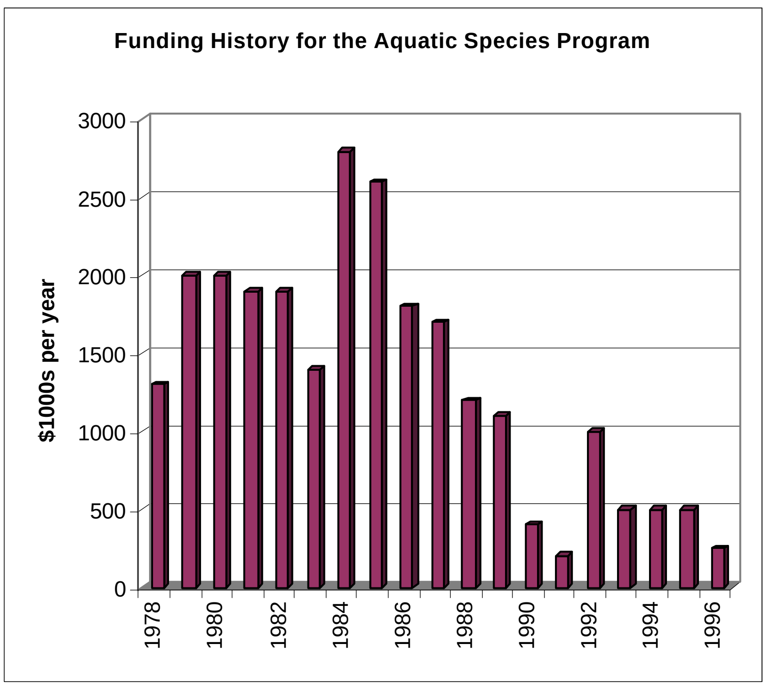
Two types of systems analysis were conducted frequently over the course of the program—resource assessments and engineering design/cost analyses. The former addresses the following important question: how much impact can algae technology have on petroleum use within the limits of available resources? Engineering designs provide some input to this question as well, since such designs tell us something about the resource demands of the technology. These designs also tell us how much the technology will cost.

As early as 1982, the program began to study the question of resource availability for algae technology. Initial studies scoped out criteria and methodology that should be used in the assessment. In 1985, a study done for Argonne National Lab produced maps of the southwestern U.S. which showed suitable zones for algae production based on climate, land and water availability. In 1990, estimates of available $CO_2$ supplies were completed for the first time. These estimates suggested that that there was enough waste $CO_2$ available in the states where climate conditions were suitable to support 2 to 7 quads of fuel production annually. The cost of the $CO_2$ was estimated to range anywhere from \$9 to \$90 per ton of $CO_2$. This study did not consider any regionally specific data, but drew its conclusions from overall data on $CO_2$ availability across a broad region. Also in 1990, a study was funded to assess land and water availability for algae technology in New Mexico. This study took a more regionally specific look at the resource question, but did so by sacrificing any consideration of available $CO_2$ supplies. This last study sums up the difficulties faced in these types of studies. The results obtained on resource availability are either able to provide a complete, but general, perspective on resources or they are more detailed in approach, but incomplete in the analysis of all resources.

Engineering design and cost studies have been done throughout the course of the ASP, with ever increasing realism in the design assumptions and cost estimates. The last set of cost estimates for the program was developed in 1995. These estimates showed that algal biodiesel cost would range from $1.40 to $4.40 per gallon based on current and long-term projections for the performance of the technology. Even with assumptions of $50 per ton of $CO_2$ as a carbon credit, the cost of biodiesel never competes with the projected cost of petroleum diesel.

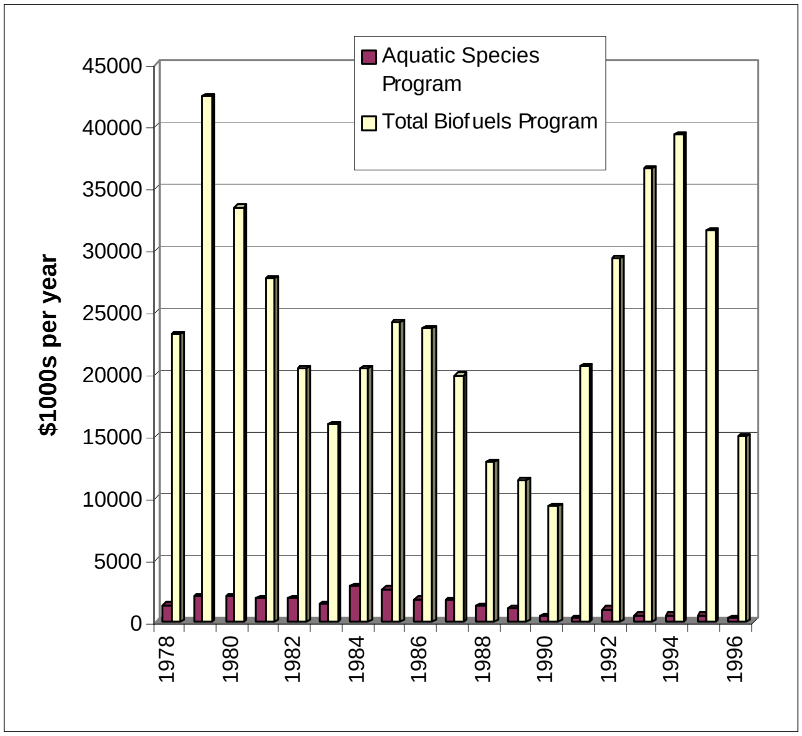
## Program Funding History

*Like all of the renewable fuels programs, the ASP has always been on a fiscal roller coaster*




In its heyday, this program leaped to levels of $2 to $2.75 million in annual funding. In most cases, these peaks came in sudden bursts in which the funding level of the program would double from one year to the next. After the boom years of 1984 and 1985, funding fell rapidly to its low of $250,000 in 1991. The last three years of the program saw a steady level of $500,000 (not counting FY 1996, which were mostly used to cover the cost of employee terminations). Ironically, these last three years were among the most productive in the history of the program (given the breakthroughs that occurred in genetic engineering). Though funding levels were relatively low, they were at least steady—providing a desperately needed stability for the program. The years of higher spending are, for the most part, dominated by costly demonstration work (the tests carried out in California, Hawaii and culminating in New Mexico), engineering analysis and culture collection activities.

*High Return for a Small Investment of DOE Funds*




The total cost of the Aquatic Species Program is $25.05 million over a twenty-year period. Compared to the total spending under the Biofuels Program ($458 million over the same period), this has not been a high cost research program. At its peak, ASP accounted for 14% of the annual Biofuels budget; while, on average, it represented only 5.5% of the total budget. Given that relatively small investment, DOE has seen a tremendous return on its research dollars.

## Future Directions

*Put less emphasis on outdoor field demonstrations and more on basic biology*

Much work remains to be done on a fundamental level to maximize the overall productivity of algae mass culture systems. The bulk of this work is probably best done in the laboratory. The results of this program's demonstration activities have proven the concept of outdoor open pond production of algae. While it is important to continue a certain amount of field work, small scale studies and research on the basic biological issues are clearly more cost effective than large scale demonstration studies.

*Take Advantage of Plant Biotechnology*

We have only scratched the surface in the area of genetic engineering for algae. With the advances occurring in this field today, any future effort on modifying algae to increase natural oil production and overall productivity are likely to proceed rapidly. The genetic engineering tools established in the program serve as a strong foundation for further genetic enhancements of algae.

*Start with what works in the field*

Select strains that work well at the specific site where the technology is to be used. These native strains are the most likely to be successful. Then, focus on optimizing the production of these native strains and use them as starting points for genetic engineering work.

*Maximize photosynthetic efficiency.*

Not enough is understood about what the theoretical limits of solar energy conversion are. Recent advances in our understanding of photosynthetic mechanisms at a molecular level, in conjunction with the advances being made in genetic engineering tools for plant systems, offer exciting opportunities for constructing algae which do not suffer the limitations of light saturation photoinhibition.

*Set realistic expectations for the technology*

Projections for future costs of petroleum are a moving target. DOE expects petroleum costs to remain relatively flat over the next 20 years. Expecting algal biodiesel to compete with such cheap petroleum prices is unrealistic. Without some mechanism for monetizing its environmental benefits (such as carbon taxes), algal biodiesel is not going to get off the ground.

*Look for near term, intermediate technology deployment opportunities such as wastewater treatment.*

Excessive focus on long term energy displacement goals will slow down development of the technology. A more balanced approach is needed in which more near term opportunities can be used to launch the technology in the commercial arena. Several such opportunities exist. Wastewater treatment is a prime example. The economics of algae technology are much more favorable when it is used as a waste treatment process and as a source of fuel. This harks back to the early days of DOE's research.

## Footnotes

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